

Application Note: Creatinine In Blood Using Capitainer®

Purpose & Background

This document provides a comprehensive overview of analytical approaches for measuring creatinine levels on standard clinical chemistry platforms using samples collected with Capitainer® whole-blood cards. It describes the laboratory workflow and highlights key considerations and precautions that must be taken into account.

Creatinine is a small nitrogen-containing molecule produced as the final, non-enzymatic breakdown product of creatine and phosphocreatine in skeletal muscle. Creatine functions as a rapid energy buffer in muscle tissue, and a small proportion of it (approximately 1–2% per day) spontaneously degrades to creatinine at a relatively constant rate proportional to total muscle mass.

Because creatinine production is relatively stable within an individual, and its clearance depends largely on renal filtration, blood creatinine concentration has long been used as a surrogate marker of kidney function. Although the glomerular filtration rate (GFR) is the best overall measure of renal function, direct measurement is complex. Serum creatinine therefore serves as an indirect filtration marker and is used to calculate an estimated GFR (eGFR), which adjusts for non-renal determinants.

Chronic kidney disease (CKD) is a common condition with an estimated prevalence of 10% (100 million adults) in Europe [1] and 14% (35.5 million adults) in the United States [2]. It is estimated that roughly two-thirds of individuals with CKD in Western countries remain undiagnosed.

Creatinine test is used for:

- *Diagnosis and monitoring of chronic kidney disease (CKD)*
- *Monitoring kidney health in conditions where CKD risk is elevated, such as diabetes, obesity, hypertension, and certain autoimmune disorders*
- *Assessing kidney function prior to radiological procedures involving contrast media*



Capitainer offers cards designed for whole blood, separated blood, and urine sampling. All products have potential applications in CKD, but this application note focuses specifically on whole-blood solutions.

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Laboratory Workflow for Capitainer® Samples

This section describes the workflow followed when a Capitainer® sampling card arrives at the laboratory. To enable analysis, the dried blood matrix must first be transferred into tubes or plate wells. The sample is then reconstituted into liquid form so that it can be processed on clinical chemistry analyzers for creatinine measurement.

Sample card handling:

To begin the reconstitution process, transfer the Capitainer® sample disc—containing the volumetric dried blood sample—into a suitable tube or plate well.

Accessing the Sample Disc

- Remove the protective tabs on the back of the Capitainer® card to expose the sample discs.
- Only one of the two sample discs on the card is required for analysis.

Transfer Methods

- **Manual Transfer:** Use tweezers or the MH1t manual card handler for precise handling.



- **Automated Transfer:** For high-throughput workflows, consider automated solutions and 96-well plate formats to streamline processing.

Proper handling of the sample disc is essential to ensure accurate sample preparation for downstream analysis.



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Aqueous versus organic extraction of dried samples:

Simplified, the process of converting dried samples back into liquid form can be divided into two main approaches:

- Aqueous reconstitution or extraction, with or without detergents
- Extraction using organic solvents, which also induce protein precipitation

Aqueous extraction is used to recover most analytes and is always required for larger molecules such as proteins. This approach yields a fully hemolyzed whole-blood sample, characterized by a red color due to hemoglobin. In contrast, organic-solvent extraction causes all proteins within the Capitainer sample disc to denature and bind to the paper matrix, while creatinine, metabolites, and other small molecules are released into a clear, protein-depleted eluate.

These differences have important implications for creatinine analysis. As described above, both creatinine assay methods detect absorbance at wavelengths that overlap with the hemoglobin absorbance peak, creating background interference in aqueous extracts.

In aqueous extracts, hemoglobin interference limits the resolution and linearity of creatinine measurements within the normal concentration range (below 100 $\mu\text{mol/L}$). However, at pathological concentrations ($>100 \mu\text{mol/L}$), the interference becomes negligible compared to the true signal, allowing excellent analytical performance for monitoring elevated creatinine levels. By contrast, the organic-solvent extract produces a matrix cleaner than serum, allowing detection of creatinine down to the assay's lowest limit of quantification.

The potential variations and considerations for optimizing extraction of dried blood samples are extensive and fall outside the scope of this application note.

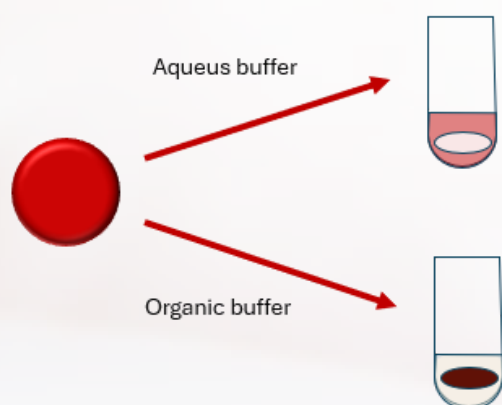


Figure 1: Using an aqueous buffer produces a red eluate and leaves the sample disc white, as all proteins are released into the buffer. In contrast, when an organic solvent is used, the sample disc remains red while only small molecules are extracted into the liquid



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Extraction protocol for creatinine

This recommended protocol is based on organic-solvent extraction using a Capitainer®B50 card:

1. Transfer one sample disc into a 2–5 mL round-bottom tube.
2. Add 250 μ L of an 80:20 methanol-to-water solution.
3. Incubate at room temperature for 60 minutes under strong agitation on an orbital shaker (approximately 700 RPM, depending on the shaker model).
4. Remove the sample disc from the tube, or aspirate the eluate and transfer it into a sample cup compatible with your analytical instrument.

Note: For Capitainer®B10 cards, 100–150 μ L of the 80:20 methanol-to-water solution is recommended. This results in a slightly higher dilution factor and a higher lower limit of quantification. An aqueous extraction using 250–500 μ L of PBS may also be applied to a B50 sample disc; however, as mentioned earlier, aqueous extraction yields poor performance at creatinine concentrations below 100 μ mol/L.

Analysis & result Calculation

The extraction protocol described above was applied in an experiment involving 25 patient samples obtained from a hospital. These samples were selected based on their known plasma creatinine concentrations, and matched EDTA whole-blood samples from the same collection event were applied to Capitainer®B50 cards. The Capitainer® B50 device collects and meters 50 μ L of blood with high precision from a finger-prick, enabling at-home sampling for creatinine measurement. For validation and comparison purposes in this study, collected EDTA blood was used instead of fingerstick samples.

EDTA whole-blood samples were applied to the Capitainer cards, dried overnight, extracted using the methanol based protocol described above, and analyzed on day one. The results were compared with the corresponding routine plasma creatinine measurements. The analytical platform used was the enzymatic creatinine method on the cobas Pro system, which has a stated measuring interval of 5–2700 μ mol/L and reports values below 5 μ mol/L with a flag.



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Note that results obtained from the extract are expressed as “ μmol creatinine per volume unit of Capitainer extract,” which differs from standard plasma units. To convert extracted values to plasma-equivalent concentrations, a correction factor must be applied. In this study, a single-point correction was performed using a data point in the middle of the dataset (original plasma creatinine = $110 \mu\text{mol/L}$). A correction factor of 4.6 was derived from this reference point and subsequently applied to all other datapoints.

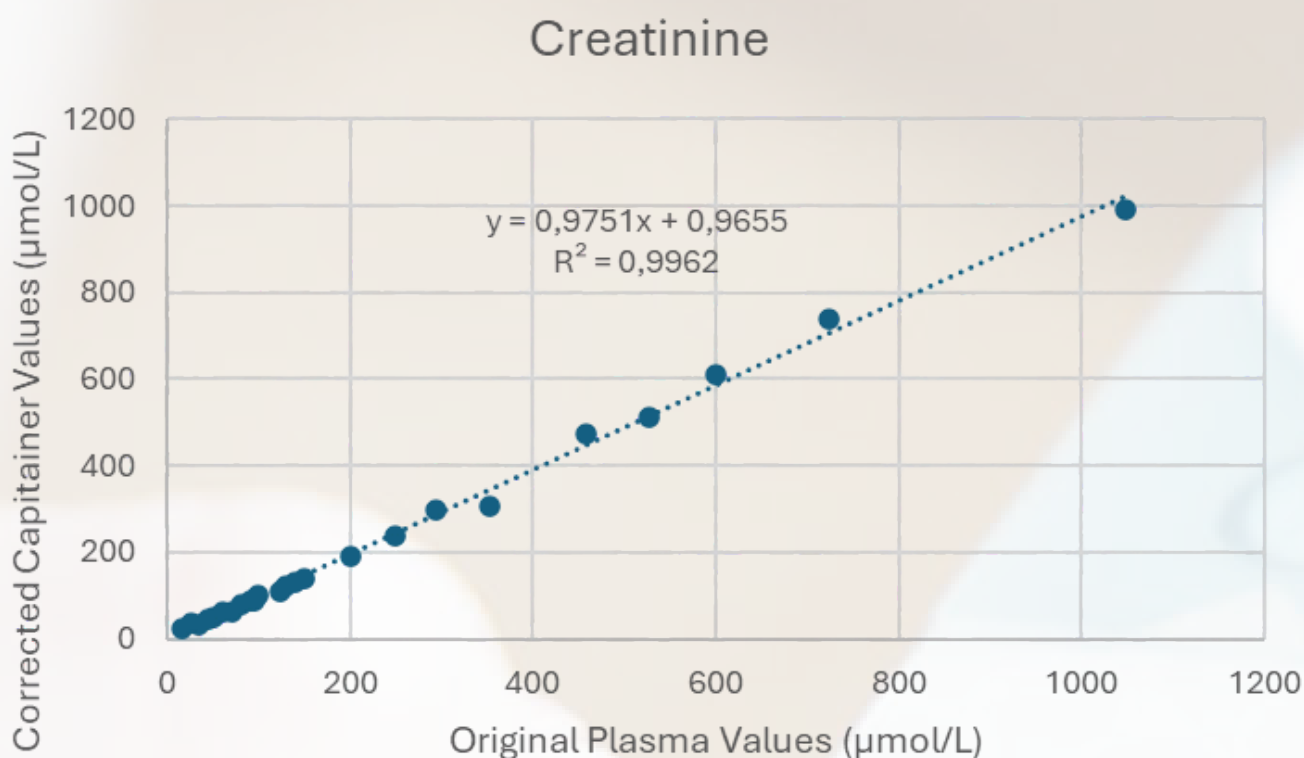


Figure 2: Linear regression comparing creatinine values from liquid samples (x-axis) and Capitainer extracts. Capitainer extract values are shown after applying the single-point correction factor.



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Accuracy, Precision and sample stability

A difference plot evaluating analytical accuracy demonstrates that the method remains robust down to approximately 30 µmol/L of creatinine. The percentage difference is elevated for the two lowest data points, with original plasma values of 17 µmol/L and 26 µmol/L. However, for values above this range, all measurements fall comfortably within the acceptance criteria. Given the intended use cases described earlier, the clinical relevance of increased variation below 30 µmol/L is minimal, if present at all.

It is also important to note that the correction factor applied in this evaluation was based on a single-point adjustment, which does not account for potential differences in where the regression line intersects the origin. Using a correction equation derived from an independent dataset—such as a linear regression of the form $Y = kx + m$ —could potentially improve performance in the very low concentration range, but this was deemed unnecessary for the purposes of this study.

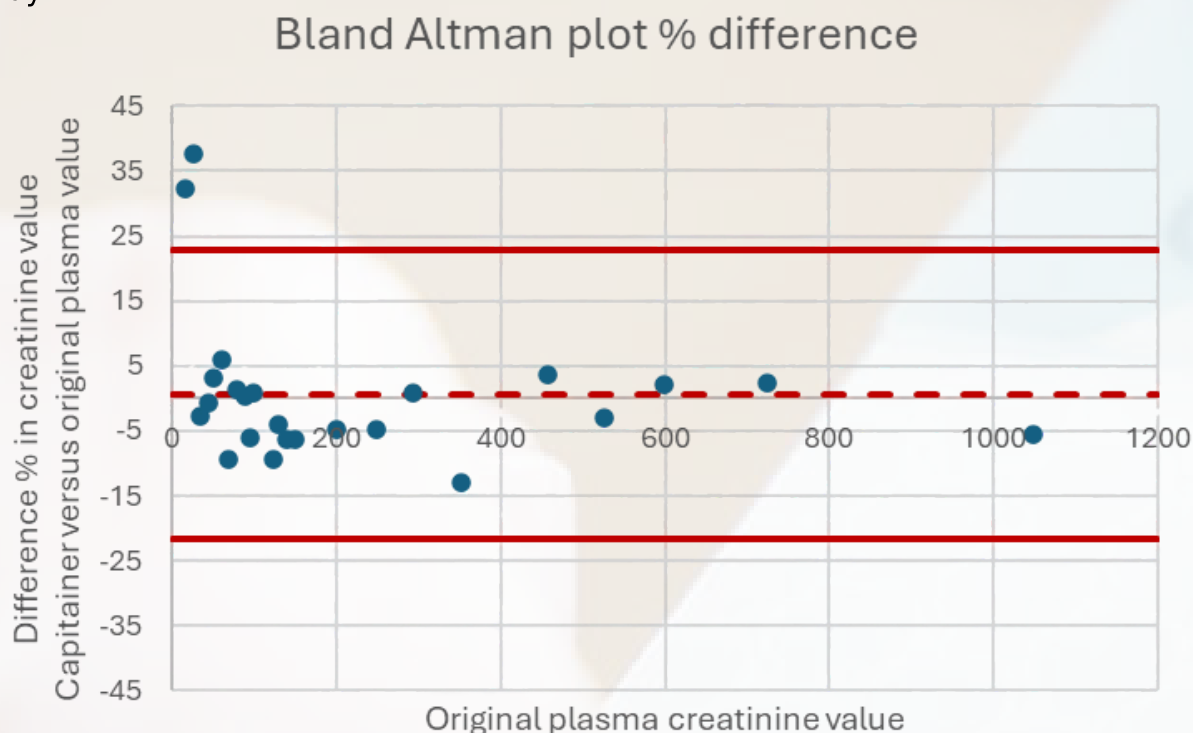


Figure 3: Difference plot showing the percentage difference between original plasma values and corrected Capitainer® B50 eluate values. The central dashed line represents the mean difference (+0.61 bias), while the upper and lower dashed lines indicate the limits of agreement (± 1.96 SD). The method is robust down to 30 µmol/L. The two lowest values, 17 and 26 µmol/L, display an increased positive bias and trigger low-level alarms on the instrument.



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The precision of the method described above—extracting Capitainer®B50 cards in 250 µL of 80:20 methanol-to-water—was evaluated by analyzing six independent sample discs and their corresponding extracts from patient samples at three different creatinine concentrations. The results confirmed good precision across all levels, including the lower concentration range.

Original plasma creatine value	Number of replicates	CV% Capitainer® extract values
34 µmol/L	6	7,83
50 µmol/L	6	7,33
100 µmol/L	6	3,64

Table 1: Repeated testing of individual sample discs and their corresponding extracts demonstrate good precision across three creatinine levels. Imprecision is slightly higher at the lowest concentrations, while precision is very high around 100 µmol/L—an analytically and clinically important range.

This experiment was also repeated on smaller sample sets to assess sample stability. The Capitainer cards provide good stability, as the dried sample discs remain protected within the card. No significant differences in results were observed for samples stored at room temperature for up to 30 days.

Aqueous extraction

As noted earlier, aqueous extraction is also an option. When hospital blood samples were analyzed using the cobas® Pro enzymatic creatinine assay following extraction with a PBS buffer, a good overall correlation was observed across a wide measurement range. However, variability in the lower concentration range was substantial, and results did not meet acceptance criteria for creatinine values below 100 µmol/L.



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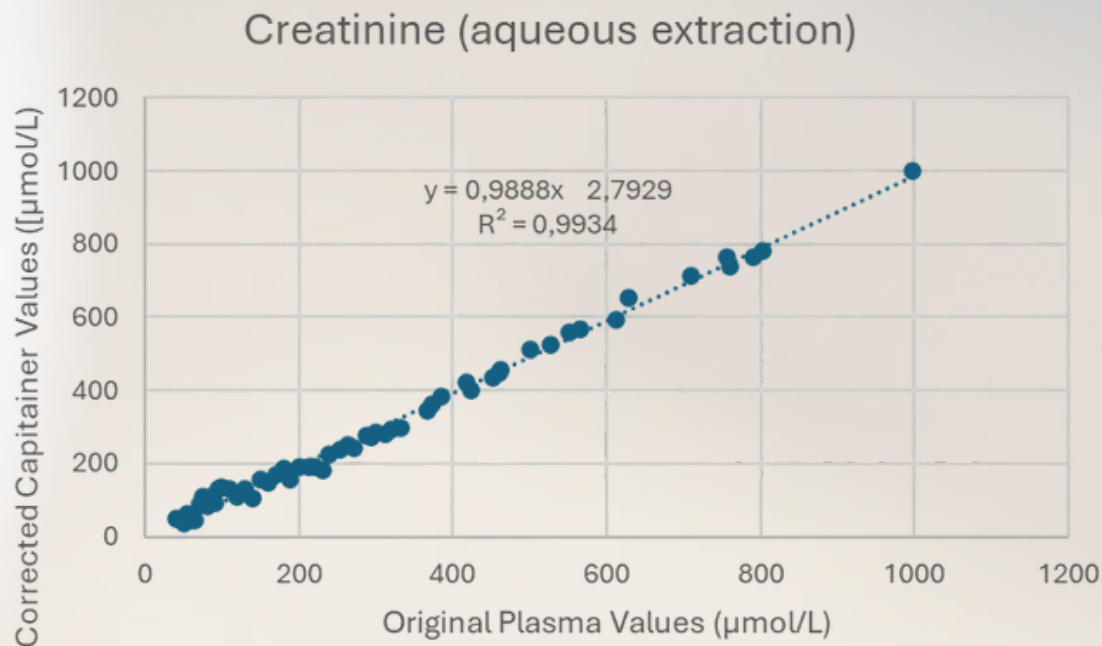


Figure 4: Fifty-five hospital blood samples were analyzed using the cobas® Pro enzymatic creatinine assay both as liquid samples and again one day after application to Capitainer® cards. A matrix-correction factor derived from a previous dataset was applied to the raw extracted values. The aqueous extract shows excellent overall correlation across the wider measurement range.

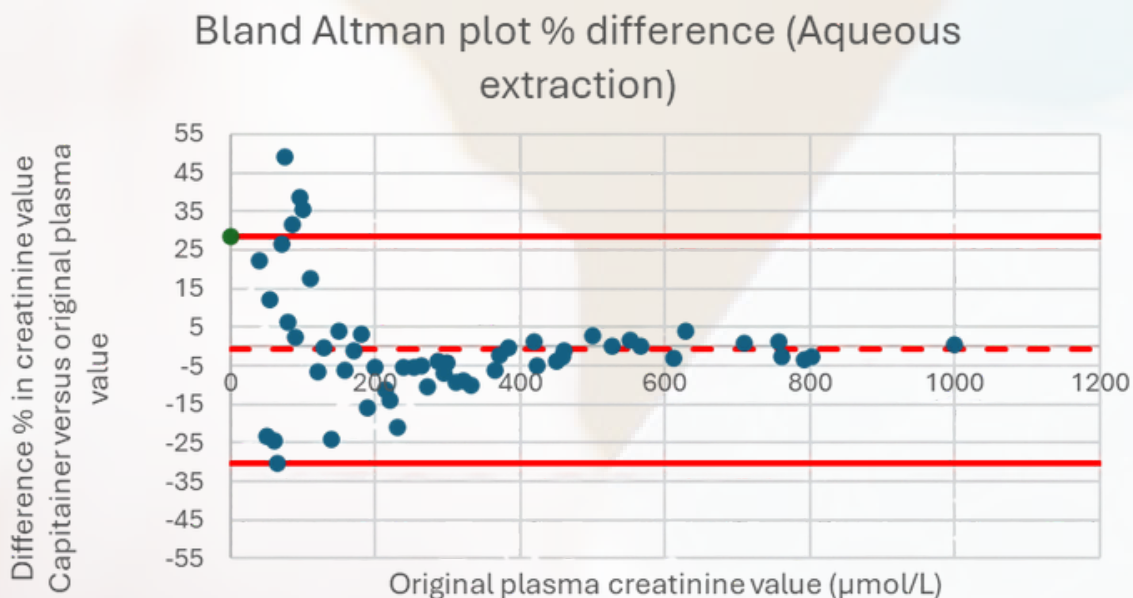


Figure 5: Difference plot showing the percentage difference between original plasma values and corrected Capitainer®B50 eluate values. The central dashed line represents the mean difference (+0.61 bias), while the upper and lower dashed lines indicate the limits of agreement (± 1.96 SD).



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However, an aqueous extraction approach may offer advantages when broader analytical panels are required. If creatinine analysis is to be combined with the measurement of additional proteins from the same extracted material, aqueous extraction becomes necessary. Alternatively, the two sample discs within the Capitainer card can be used in parallel—one processed with organic extraction and the other with aqueous extraction.

It should be noted that clearly pathological creatinine concentrations above 100 $\mu\text{mol/L}$ can still be reliably detected using the aqueous approach, although resolution in the normal concentration range is not sufficient for precise quantification.

Summary

This application note demonstrates that creatinine can be measured with high accuracy and analytical confidence from samples collected using Capitainer® cards in combination with standard enzymatic clinical chemistry assays. The inherently quantitative design of the Capitainer system—where microfluidic metering ensures an exact and highly reproducible blood volume—provides an exceptionally reliable sample matrix for precise creatinine determination.

Key findings include:



Organic extraction using 80:20 methanol-to-water delivers a robust and highly consistent method across a wide analytical range of 30–1000 $\mu\text{mol/L}$.



Aqueous extraction remains a viable option, although the usable range is more limited, reliably covering 100–1000 $\mu\text{mol/L}$.

Methanol-based extraction is preferred, as it produces a hemoglobin-free eluate, thereby eliminating the optical interference that limits accuracy in the lower concentration range.

A noted limitation of this study is that capillary finger-prick samples were not included. However, previously published work has already demonstrated excellent agreement between venous and capillary creatinine measurements, with no clinically meaningful differences reported [3].



Taken together, these results strongly support the suitability of Capitainer cards for decentralized and at-home creatinine testing, offering both analytical performance and practical usability that align well with modern clinical needs.



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References

1. ([Prevalence, outcomes, and cost of chronic kidney disease in a contemporary population of 2·4 million patients from 11 countries: The CaReMe CKD study - The Lancet Regional Health - Europe](#))
2. ([Kidney Disease Statistics for the United States - NIDDK](#))
3. Woolley T, Rutter E and Staudenmaier M (2023) Comparability and Stability of Serum Creatinine Concentration in Capillary and Venous Blood. Br J Biomed Sci 80:11402. doi: 10.3389/bjbs.2023.11402

